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IFN-γ induces apoptosis in mouse embryonic stem cells, a putative mechanism of its embryotoxicity

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It has been reported that interferon (IFN)-γ should inhibit *in vitro* mouse embryo growth by direct cell toxicity. However, the mechanism involved has not been clearly established. In the present study, this question was addressed using the embryonic stem (ES) cell model. It was found that IFN-γ induces a dose-dependent apoptosis in ES cells, as assessed by trypan-blue staining, by Annexin-V labeling and DNA analysis. Moreover, IFN-γ treatment cooperates with Fas-mediated apoptosis, a phenomenon that has been recently reported. As Bcl-2 oncoprotein functions as a death repressor molecule in an evolutionarily conserved cell death pathway, its expression was analyzed by flow cytometry. It was demonstrated that Bcl-2 is expressed in ES cells. When compared to untreated ES cells, IFN-γ-treated, apoptotic cells expressed a lower Bcl-2 level and a normal level of Fas, whereas surviving cells expressed a normal level of Bcl-2 but a lower Fas expression. Altogether, these data suggest that IFN-γ may influence early mouse embryo development by promoting apoptosis, which may constitute a novel mechanism of IFN-γ embryotoxicity.

Key words: apoptosis, bcl-2, embryonic stem cells, IFN-γ.

Introduction

Apoptosis is as fundamental to cellular and tissue physiology as cell division and differentiation (Granville *et al.* 1998). It is an essential feature of normal physiology in a variety of organs and it plays an important role in fetal development (Lea *et al.* 1997). The well-defined loss of specific cells is crucial during embryonic development as a part of organogenesis (Gluksman 1951). There is also increasing evidence that regulated apoptosis is important during implantation and early pregnancy. Thus, an unresolved developmental question is how a cell knows whether it should survive or self-destruct. Determining the expression pattern of proteins that regulate cell survival is a critical aspect of understanding this process.

Interferon (IFN)-γ has been shown to be toxic to embryonic and trophoblast cells *in vitro* (Hill *et al.* 1987; Berkowitz *et al.* 1988; Haimovici *et al.* 1991), and it may play a role in reproductive dysfunction in unexplained recurrent abortion (URA) patients (Hill *et al.* 1995). Recently, IFN-γ was reported not to impair early embryo development, but to significantly inhibit blastocyst spreading (Cameo *et al.* 1999). γ-Interferon is able to induce apoptosis in various cells, such as vascular smooth muscle cells, normal endothelial cells and differential leukemic B-cell lines (Trubiani *et al.* 1994). IFN-γ inhibited HT29 human adenocarcinoma cell growth through Fas-mediated apoptosis, and STAT1 protein is required to up-regulate Fas and FasL expression during this procedure (Xu *et al.* 1998). γ-Interferon also activated Fas-mediated apoptosis in IL-6-dependent and IL-6-independent multiple myeloid cell lines through up-regulation of Fas antigen expression but did not alter the expression of Bcl-2 or Bax (Spets *et al.* 1998). However, there was also a report that IFN-γ inhibited apoptosis induced by wild-type p53, indicating that it could be an anti-apoptotic cytokine for myeloid cells in which apoptosis was induced by wild-type p53, cytotoxic anticancer agents or viability factor deprivation (Lotem & Sachs 1995). γ-Interferon in the culture suppressed human mast cell apoptosis and prolonged their survival in a dose-dependent manner (Yanagida *et al.* 1996). Thus these different results may demonstrate that the IFN-γ-mediated apoptosis is cell type dependent.

Bcl-2 was initially discovered as an overexpressed

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